

Teratogenic Effects of Selenium in Natural Populations of Freshwater Fish

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The prevalence of abnormalities and associated tissue selenium residues were assessed for the fish population of Belews Lake, North Carolina, and two reference lakes in 1975, 1978, 1982, and 1992. Teratogenic defects identified included lofdosis, kyphosis, scoliosis, and head, mouth, and fin deformities. Many fish exhibited multiple malformations and some were grossly deformed and distorted in appearance. Other abnormalities observed were edema, exophthalmus, and cataracts. Whole-body tissue residues of selenium in the fishes of Belews Lake were up to 130 times those in the reference lakes and the incidence of abnormalities was some 7 to 70 times greater. Teratogenic defects increased as selenium levels rose between 1975 and 1982 and fell with declining selenium levels between 1982 and 1992 as selenium inputs into Eklews Lake were curtailed. The relationship between selenium residues and prevalence of malformations approximated an exponential function ($R^2 = 0.88$, $P < 0.01$; cubic model) for centrarchids over the range of 1-80 $\mu\text{g/g}$ dry wt selenium and 0-70% deformities. This relationship could be useful in evaluating the role of teratogenic effects in warm-water fish populations suspected of having selenium-related reproductive failure. Unique conditions may have existed in Belews Lake which led to the high frequency and persistence of deformities in juvenile and adult fish. In other, less-contaminated locations competition and predation may eliminate malformed individuals in all but the larval life stage. Teratogenesis could be an important, but easily overlooked phenomenon contributing to fishery reproductive failure in selenium-contaminated aquatic habitats. © 1993 Academic Press Inc

INTRODUCTION

Selenium has been reported to cause several types of developmental abnormalities in birds, mammals, and fish. As early as 1935, poor hatching success and deformities in chick embryos were linked to high levels of selenium in the diet of laying hens (Franke and Tulley, 1935). Subsequent experimental studies confirmed the teratogenic effects of inorganic selenium salts and organic selenium compounds on chick embryos (Franke *et al.*, 1936; Halverson *et al.*, 1965; Palmer *et al.*, 1973). The most common deformities were abnormal development of the legs, feet, and beak. In the mid 1980s, laboratory and field studies confirmed that selenium in agricultural irrigation drainwater caused extensive teratogenesis in wild populations of aquatic birds in central California (Ohlendorf *et al.*, 1986, 1989; Presser and Ohlendorf, 1987; Heinz *et al.*, 1987; Ohlendorf, 1989). The deformities were often multiple and included anomalies in the brain, heart, liver, and skeleton as well as missing or abnormal eyes, beaks, wings, legs, and feet (Kilness and Simmons, 1986; Ohlendorf *et al.*, 1986, 1988; Hoffman and Heinz, 1988; Hoffman *et al.*, 1988).

In mammals, the offspring of sheep, rats, and pigs all exhibit malformations following administration of excessive selenium during pregnancy. Deformities of the feet, legs, hooves, ears, eyes, and skeleton have been observed in experimental studies (Rosenfeld

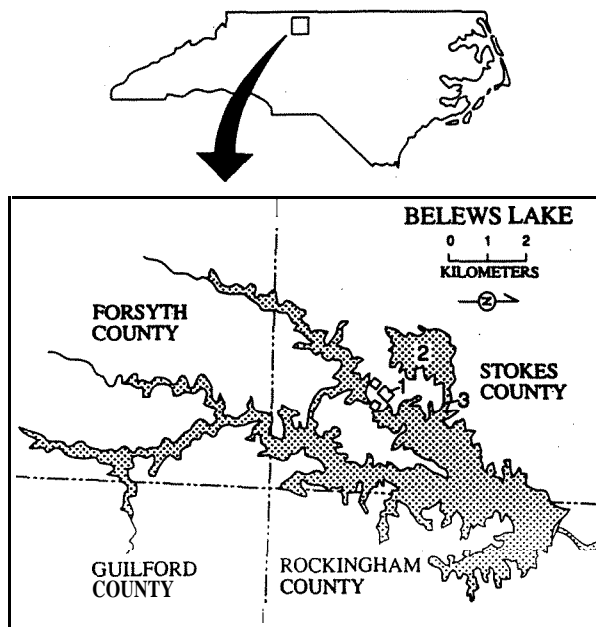


FIG. 1. Geographic location of Belews Lake, North Carolina. 1, Belews Creek Steam Station; 2, ash settling basin; 3, ash basin return water canal.

and Beath, 1947, 1954; Wahlstrom and Olson, 1959). Similar teratogenic conditions have occurred in the young of livestock grazing on seleniferous rangelands (Rosenfeld and Reath, 1964). Selenium has even been implicated as a possible human teratogen (Robertson, 1970).

Selenium has been shown to be teratogenic to fish in laboratory and field reproductive studies. Deformed young resulted when parents were given selenomethionine in the diet (Woock *et al.*, 1987) or held in solutions of sodium selenate (Pyron and Beitingger, 1989) prior to spawning, when larval fish were reared in solutions of sodium selenite (Goettl and Davies, 1976, 1977) or selenium dioxide (Niimi and LaHam, 1975), when experimental streams containing adult fish were dosed with sodium selenite (Schultz and Hermanutz, 1990; Hermanutz, 1992; Hermanutz *et al.*, 1992), or when adult fish were removed from a contaminated field site and spawned artificially in the laboratory (Bryson *et al.*, 1984; Gillespie and Baumann, 1986). Types of deformities included distended pericardial and abdominal cavities, lordosis (concave curvature of lumbar region of spine), scoliosis (lateral curvature of spine), kyphosis (convex curvature of thoracic region of spine resulting in humpback), misshaped heads and mouths, and distorted caudle peduncles. Although these reproductive studies indicate that selenium is teratogenic to fish, they did not determine if terata occur or persist in reservoir fish populations exposed to high levels of selenium. Moreover, the assumption has been made that teratogenic effects would be lethal before the fish grew to adulthood since deformed larval fish observed in experimental studies usually die prior to the swim-up stage (Gillespie and Baumann, 1986; Woock *et al.*, 1987; Pyron and Beitingger, 1989; Schultz and Hermanutz, 1990; Hermanutz, 1992; Hermanutz *et al.*, 1992).

During the 1970s, selenium present in wastewater from a coal-fired power plant in North Carolina accumulated in the adjacent cooling-water reservoir (Belews Lake) and led to mortality and reproductive failure in the fish population (Cumbie and Van Horn, 1978). Concentrations of selenium in fish tissues ranged from 20 to 125 $\mu\text{g/g}$ (dry weight) and were 10 to 100 times higher than those from nearby reference sites (Lemly, 1985a). This episode of contamination provided a unique opportunity to assess the long-term environmental 'impact of selenium in an aquatic ecosystem. Moreover, the discharge of selenium from the power plant into the reservoir was curtailed in 1985, providing an opportunity to assess recovery of the fishery from toxic conditions. The author began studies to monitor and evaluate the cycling and toxicity of selenium in Belews Lake starting with the initial operation of the power plant in 1974. The results of intensive studies of selenium residues and associated reproductive effects on the fishery were reported previously (Lemly, 1985a,b). From the earliest portions of these investigations, it was apparent that there was a high incidence of deformities present in the fish population. Four surveys were conducted of the frequency and types of abnormalities, and associated selenium residues in tissues, occurring in the fish present in Belews Lake and two reference sites from 1975 to 1992. Three of them (1975, 1978, 1982) were made while the power plant was discharging selenium-laden wastewater into the reservoir but the last (1992) was conducted some 7 years after these aqueous discharges had been curtailed. This is the first report of the prevalence and persistence of selenium teratogenesis in natural populations of fish.

MATERIALS AND METHODS

Study Area

Belews Lake is a Duke Power Company impoundment situated on Belews Creek, a small tributary to the Dan River, located in the northwestern Piedmont area of North Carolina (Fig. 1). At the dam site, the drainage area of the Belews Creek watershed is approximately 30,000 hectares (76 square miles). Construction of the reservoir began in spring 1970 and the lake reached full pool elevation in April 1973, somewhat ahead of the design schedule. This early filling of the lake allowed over 1 year of environmental stabilization to occur before the first generating unit of the Belews Creek Steam Station went on-line. At full pool, Belews Lake has a surface area of approximately 1560 hectares, a shoreline of 110 km, a maximum depth of 42 m, and a mean depth of 14 m (Weiss and Anderson, 1978).

Belews Lake was constructed to provide a source of condenser cooling water for the Belews Creek Steam Station, the largest coal-fired electric-generating station on the Duke Power system and one of the largest in the United States. The station has two generating units; the first began operating in August 1974, the second in December 1975. Each of the two units has a generating capacity of 1140 MW and can consume up to 500 tonnes of coal per hour. Cooling water is supplied to each unit from Belews Lake at a maximum rate of 35 m^3/sec (2500 cfs), which is sufficient to circulate the entire lake volume through the power plant every 37 days. The average flow into the lake from the drainage basin is approximately 2.6 m^3/sec ; however, due to forced evaporation from the heated discharge from the Belews Creek Steam Station the average outflow is only 2.1 m^3/sec . Consequently, the average volume replacement time of Belews Lake increased from about 1000 days to 1500 days when the power plant began operating (Cumbie, 1978).

From 1974 through 1985, **water** was withdrawn from the lake and mixed with bottom ash from the coal burners and fly ash collected by electrostatic precipitators. This slurry was pumped from the power plant and discharged into a **142-hectare** ash basin, where suspended solids were collected by gravitational settling. Runoff water from the coal storage area and power plant site was collected by sump units and also pumped into the ash **basin**. Selenium-laden (150-200 $\mu\text{g/liter}$) return flows **from** the ash basin entered the west side of Belews Lake through an ash sluice water canal (Fig. 1). In response to selenium-induced problems with the fishery of Belews Lake, Duke Power Company switched to a dry-ash handling system in December 1985 (North Carolina Department of Natural Resources and Community Development, 1986). Fly ash is now disposed in a landfill on the power plant site. Bottom ash and stormwater runoff from the landfill are conveyed into the old ash basin, which has been diverted to drain into the nearby Dan River.

Collection and Examination of Fishes

A survey of the externally visible abnormalities present in the fish population of Belews Lake and two nearby reference sites (High Rock Lake, Davidson County, NC; **Badin** Lake, Montgomery County, NC) was conducted in 1975, 1978, 1982, and 1992. The fish were collected with seines, **dipnets**, and traps. All fish were identified and enumerated, and the normal individuals were released unharmed except for those that were kept for selenium residue analysis. Deformed individuals were placed on ice and returned to the laboratory for closer inspection and identification of multiple malformations. Five major categories were used to classify the abnormalities: (1) spinal deformities (lordosis, concave curvature of lumbar and **caudal** regions of spine; **scoliosis**, lateral curvature of spine; kyphosis, convex curvature of thoracic region of spine resulting in humpback), (2) accumulation of body fluid (edema, distended abdominal cavity; exophthalmus, fluid behind eyeball resulting in protruding eyes or "popeye"), (3) missing or abnormal fins, (4) abnormally shaped head or mouth, (5) cloudy eye lens or cornea (cataracts). Spinal deformities were examined using X-ray images to confirm the specific types of abnormalities as well as their severity.

Selenium Residue Analysis

For each survey, three to five normal individuals of each fish species were combined to yield a composite tissue sample for whole-body selenium determinations. Six to ten individuals were composited for small minnows and **forage** fishes in order to supply sufficient sample mass for analysis. The same number of individuals were used to generate composite samples of abnormal fish (except 1992 Belews Lake samples, which were formulated using all individuals collected) and they were selected to **include** as many of the five major categories of abnormalities as possible. Whole fish were homogenized in a blender (large fish were cut into sections first) and then separated into two subsamples and frozen at -40°C until residue analyses were performed (usually within 30 days). Specimens and samples were manipulated with linear **polyethylene** (LPE), polypropylene, or stainless-steel instruments during collection, preparation, and analysis.

Concentrations of selenium were measured by means of differential pulse **polarography** (DPP) using a Princeton Applied Research Model 174-A Polarographic Analysis System, by flameless atomic absorption (FAA) with a Scintrex **Model AAZ-2**

Zeeman modulated spectrophotometer, or by hydride atomic absorption (HAA) using a Perkin-Elmer Model 5000 spectrophotometer and VGA-76 vapor generation unit. Operating conditions for these instruments followed procedures given by Brown (1977), Lowry (1978), Lemly (1982), Bodenseewerk Perkin-Elmer and Company (1977), and Brumbaugh and Walther (1989). Smoke-free conditions were maintained at all times during sample preparation and analysis.

Quality control samples were analyzed regularly to evaluate the accuracy and precision of analytical results. The measured concentrations of selenium in National Bureau of Standards (NBS) 1577 bovine liver were $1.05 \pm 0.07 \mu\text{g/g}$ (mean \pm SD, $n = 36$) which were within the certified range of 1.0 to 1.2 $\mu\text{g/g}$. Selenium concentrations in NBS RM 50 tuna were $3.57 \pm 0.22 \mu\text{g/g}$ (mean \pm SD, $n = 36$) which were also within the certified range of 3.2 to 4.0 $\mu\text{g/g}$. Mean percentage relative standard deviation for samples that were prepared and analyzed in triplicate was 2.13 (range = 0.89 to 5.11%; $n = 24$ triplicate groups). Recovery of selenium from spiked tissue samples and homogenates averaged 96% (range = 83 to 116%). The limit of detection for selenium in tissue samples averaged 0.079 $\mu\text{g/g}$ (range = 0.019 to 0.253 $\mu\text{g/g}$). Concentrations of selenium in all procedural blank samples were at or below the corresponding limit of detection and the recovery of selenium from analytical spikes averaged 97% (range = 93 to 104%).

RESULTS

A total of 22 fish species, representing eight families, were examined for abnormalities during the four surveys of this study (Table 1). In 1975, defects were observed in all 19 species collected from Belews Lake but were generally more abundant among centrarchids (10 to 55%) than among other species (3 to 33%; Table 2). The most frequent types of abnormalities were lordosis, kyphosis, and fin defects followed by edema, cataracts, scoliosis, exophthalmus, and head deformities (Table 2). Skeletal, fin, and head defects were restricted to juvenile fish, but edema, exophthalmus, and cataracts were commonly found in adults as well. Many fish exhibited multiple malformations and were sometimes grossly deformed and distorted in appearance. Examples of the types and severity of deformities seen are shown in Figs. 2 through 7. Some of the deformities such as vestigial or missing fin rays and pughead (Figs. 2-4 and 6) were quite subtle and required close inspection to detect, while other abnormalities such as lordosis, kyphosis, scoliosis, and exophthalmus were often striking in appearance (Figs. 2, 4, 5, and 7). Almost no abnormalities were observed in the fish populations of the two reference lakes; a total of two head deformities and six fin deformities were found. Fish from Belews Lake contained whole-body concentrations of selenium ranging from 40 to 67 $\mu\text{g/g}$ (dry weight) and the residues present in normal individuals were equivalent to those in abnormal individuals (Table 3). In contrast, selenium levels in fish from the reference lakes were below 2.4 $\mu\text{g/g}$ (Table 3).

In the 1978 survey, only four fish species persisted in Belews Lake (carp, fathead minnows, black bullheads, mosquitofish). Deformities were quite common in these species and the frequency of total abnormalities reached 34% in fathead minnows (Table 2). Skeletal deformities were found in adult fish as well as in juveniles. The pattern of abnormalities was somewhat different from that observed in 1975. Although spinal and fin deformities were still the most common, there was a noticeable increase in the relative proportion of head deformities and cataracts and a reduction in the

TABLE 1
SCIENTIFIC NAMES OF FISHES MENTIONED IN FIGURES,
TABLES, AND TEXT

Scientific name ^a	Common name
Catostomidae	
<i>Catostomus commersoni</i>	White sucker
Centrarchidae	
<i>Lepomis auritus</i>	Redbreast sunfish
<i>Lepomis cyanellus</i>	Green sunfish
<i>Lepomis gibbosus</i>	Pumpkinseed sunfish
<i>Lepomis gulosus</i>	War-mouth sunfish
<i>Lepomis macrochirus</i>	Bluegill sunfish
<i>Lepomis microlophus</i>	Redear sunfish
<i>Micropterus salmoides</i>	Largemouth bass
<i>Pomoxis annularis</i>	White crappie
<i>Pomoxis nigromaculatus</i>	Black crappie
Clupeidae	
<i>Alosa aestivalis</i>	Blueback herring
<i>Dorosoma petenense</i>	Threadfin shad
Cyprinidae	
<i>Cyprinus carpio</i>	Common carp
<i>Notemigonus crysoleucas</i>	Golden shiner
<i>Notropis lutrensis</i>	Red shiner
<i>Notropis analostanus</i>	Satinfin shiner
<i>Pimephales promelas</i>	Fathead minnow
Ictaluridae	
<i>Ictalurus melas</i>	Black bullhead
<i>Ictalurus punctatus</i>	Channel catfish
Percichthyidae	
<i>Morone americana</i>	White perch
Percidae	
<i>Perca flavescens</i>	Yellow perch
Poeciliidae	
<i>Gambusia affinis</i>	Mosquitofish

^a Scientific names as given in Robins *et al.* (1980).

occurrence of edema and exoptbalmus. This apparent shift was likely due to the elimination of some planktivorous species, threadfin shad and blueback herring for example, which frequently exhibited edema as an abnormality. The prevalence of abnormalities in the reference lakes remained below 4%. Tissue residues of selenium in fish from Beleys Lake were approximately double the levels found in the 1975 survey, ranging from 80 to 132 $\mu\text{g/g}$ (Table 3). As in 1975, there was essentially no difference in residues between normal and abnormal fish. Concentrations of selenium in fish from the reference lakes had not changed from 1975 levels and remained below 2.5 $\mu\text{g/g}$ (Table 3).

Six species were collected from Beleys Lake in the 1982 survey; the four collected in 1978 along with green sunfish and red shiners. Green sunfish gradually returned to the main body of Beleys Lake from isolated headwater areas during the 1978-1982 interval and were collected in small numbers. Red shiners were accidentally introduced into the lake in 1980 by sportfishermen (baitfish release) and were a co-dominant

PREVALENCE AND TYPES OF ABNORMALITIES IN FISH EXPOSED TO ELEVATED SELENIUM IN BELEWS LAKE AS COMPARED TO THOSE IN FISH FROM REFERENCE SITES

Year and species ¹	Site ^b and number collected	Type of abnormality ^c and number with the defect							Total with abnormalities (%) ^d
		Lordosis	Scoliosis	Kyphosis	Edema	Exophthalmus	Head	Fin Eye	
1975									
White sucker	BEL-26	1	0	0	0	0	1	3	1
	HRL-31	0	0	0	0	0	0	0	0
	BAL-19	0	0	0	0	0	0	0	0
Redbreast sunfish	BEL-34	6	4	5	1	1	1	4	2
	HRL-30	0	0	0	0	0	0	0	0
	BAL-36	0	0	0	0	0	0	0	0
Green sunfish	BEL-49	12	2	15	2	1	5	9	6
	HRL-52	0	0	0	0	0	0	1	0
	BAL-37	0	0	0	0	0	0	0	0
Pumpkinseed	BEL-20	3	1	3	0	0	2	6	2
	HRL-2 I	0	0	0	0	0	0	0	0
	BAL-39	0	0	0	0	0	0	0	0
Warmouth sunfish	BEL-18	4	0	4	1	0	0	4	0
	HRL-32	0	0	0	0	0	0	1	0
	BAL-36	0	0	0	0	0	0	0	0
Bluegill sunfish	BEL-32	9	1	9	2	3	1	1	4
	HRL-46	0	0	0	0	0	0	1	0
	BAL-49	0	0	0	0	0	0	0	0
Redear sunfish	BEL-2 I	1	0	2	0	0	0	1	0
	HRL-20	0	0	0	0	0	0	0	0
	BAL-33	0	0	0	0	0	0	0	0
Largemouth bass	BEL-26	1	1	1	0	0	0	4	1
	HRL-37	0	0	0	0	0	0	0	0
	BAL-32	0	0	0	0	0	1	0	0
White crappie	BEL-3 I	4	2	4	3	6	1	2	6
	HRL-43	0	0	0	0	0	0	0	0
	BAL-29	0	0	0	0	0	0	0	0
Black crappie	BEL-17	0	0	0	0	7	0	3	5
	HRL-3	0	0	0	0	0	0	0	0
	BAL-40	0	0	0	0	0	0	0	0
Blueback herring	BEL-4 I	0	2	0	4	0	0	0	0
	HRL-35	0	0	0	0	0	0	0	0
	BAL-79	0	0	0	0	0	0	0	0
Threadfin shad	BEL-67	3	4	1	11	3	2	7	5
	HRL-118	0	0	0	0	0	0	1	0
	BAL-125	0	0	0	0	0	0	0	0
Common carp	BEL-38	1	1	1	0	0	0	0	0
	HRL-25	0	0	0	0	0	0	0	0
	BAL-25	0	0	0	0	0	0	0	0
Golden shiner	BEL-17	1	2	1	3	1	2	3	1
	HRL-30	0	0	0	0	0	0	0	0
	BAL-2 I	0	0	0	0	0	0	0	0
Black bullhead	BEL-53	1	0	1	1	0	0	1	3
	HRL-24	0	0	0	0	0	0	0	0
	BAL-2 I	0	0	0	0	0	0	1	0
Channel catfish	BEL-12	2	0	2	0	0	0	2	0
	HRL-35	0	0	0	0	0	0	0	0
	BAL-29	0	0	0	0	0	0	0	0
White perch	BEL-18	1	0	1	2	5	0	0	3
	HRL-44	0	0	0	0	0	0	0	0
	BAL-37	0	0	0	0	0	0	0	0
Yellow perch	BEL-69	0	0	0	0	0	0	2	1
	HRL-46	0	0	0	0	0	0	0	0
	BAL-32	0	0	0	0	0	0	0	0

Year and species ^a	Site ^b and number collected	Type of abnormality ^c and number with the defect								Total with abnormalities (%) ^d
		Lordosis	Scoliosis	Kyphosis	Edema	Exophthalmus	Head	Fin	Eye	
Mosquitofish	BEL-146	27	18	27	19	7	12	27	13	30 (21)
	HRL-52	0	0	0	0	0	1	0	0	1 (2)
	BAL-59	0	0	0	0	0	0	1	0	1 (2)
Total	EEL-735	78	38	79	50	29	27	77	49	153 (21)
	HRL-752	0	0	0	0	0	1	0	0	5 (1)
	BAL-778	0	0	0	0	0	1	0	0	3 (1)
1978										
Common carp	BEL-41	2	0	1	3	1	0	4	1	5 (12)
	HRL-40	0	0	0	0	0	0	0	0	0 (0)
	BAL-32	0	0	0	0	0	0	0	0	0 (0)
Fathead minnow	BEL-125	23	11	23	7	5	19	21	16	42 (34)
	HRL-67	0	0	0	0	0	0	0	0	0 (0)
	BAL-48	0	0	0	0	0	0	0	0	0 (0)
Black bullhead	BEL-34	1	1	2	0	0	2	4	1	7 (21)
	HRL-27	0	0	0	0	0	0	2	0	2 (7)
	BAL-28	0	0	0	0	0	0	0	0	0 (0)
Mosquitofish	BEL-109	12	3	11	5	4	9	10	7	29 (27)
	HRL-66	0	0	0	0	0	0	0	0	0 (0)
	BAL-74	0	0	0	0	0	0	3	0	3 (4)
Total	BEL-309	38	15	37	15	10	30	39	25	83 (27)
	HRL-200	0	0	0	0	0	0	0	0	2 (1)
	BAL-182	0	0	0	0	0	0	0	0	3 (2)
1982										
Green sunfish	BEL-37	22	1	22	3	4	2	9	8	26 (70)
	HRL-50	0	0	0	0	0	0	1	0	1 (2)
	BAL-29	0	0	0	0	0	0	1	0	1 (3)
Common carp	BEL-28	3	0	3	1	0	1	17	4	17 (61)
	HRL-27	0	0	0	0	0	0	2	0	2 (7)
	BAL-22	0	0	0	0	0	0	0	0	0 (0)
Red shiner	BEL-136	6	12	7	0	5	5	11	6	21 (15)
	HRL-21	0	0	0	0	0	0	0	0	0 (0)
	BAL-0	—	—	—	—	—	—	—	—	—
Fathead minnow	BEL-119	31	19	31	8	4	16	38	2	40 (34)
	HRL-44	0	0	0	0	0	0	1	0	1 (2)
	BAL-45	0	0	0	0	0	1	0	0	1 (2)
Black bullhead	BEL-34	6	0	6	0	0	0	6	0	6 (18)
	HRL-41	0	0	0	0	0	0	0	0	0 (0)
	BAL-31	0	0	0	0	0	0	0	0	0 (0)
Mosquitofish	BEL-140	39	6	39	18	11	6	32	10	49 (35)
	HRL-65	0	0	0	0	0	0	1	0	1 (2)
	BAL-112	0	0	0	0	0	1	3	0	4 (4)
Total	BEL-494	107	38	108	39	20	30	113	30	159 (32)
	HRL-248	0	0	0	0	0	0	5	0	5 (2)
	BAL-239	0	0	0	0	0	2	4	0	6 (3)
1992 ^e										
Green sunfish	BEL-71	1	0	1	0	0	0	7	0	8 (11)
	HRL-66	0	0	0	0	0	0	0	0	0 (0)
	BAL-50	0	0	0	0	0	0	0	0	0 (0)
Bluegill sunfish	BEL-71	0	0	0	0	0	0	3	1	4 (6)
	HRL-64	0	0	0	0	0	0	0	0	0 (0)
	BAL-69	0	0	0	0	0	0	0	0	0 (0)
Largemouth bass	BEL-21	1	0	1	0	0	0	1	0	1 (5)
	HRL-34	0	0	0	0	0	0	0	0	0 (0)
	BAL-30	0	0	0	0	0	0	0	0	0 (0)

TABLE 2-Continued

Year and species ^a	Site ^b and number collected	Type of abnormality ^c and number with the defect								Total with abnormalities (%) ^d
		Lordosis	Scoliosis	Kyphosis	Edema	Exophthalmus	Head	Fin	Eye	
Common carp	BEL-29	0	0	0	0	0	0		20	2 (7)
	HRL-28	0	0	0	0	0	0		00	0 (0)
	BAL-26	0 ^e	0	0	0	0	0		10	1 (4)
Red shiner	BEL-35	0	0	0	0	0	0	1	00	0 (0)
	HRL-30	0	0	0	0	0	0			
	BAL-41	0	0	0	0	0	0	4	1	4 (5)
Satinfin shiner	BEL-75	0	0	0	0	0	0			0 (0)
	HRL-0	0	0	0	0	0	0			0 (0)
	BAL-41	0	0	0	0	0	0			0 (0)
Fathead minnow	BEL-82	3	0	0	0	0	1	6	1	8 (10)
	HRL-49	0	0	0	0	0	0		10	1 (2)
	BAL-61	0	0	0	0	0	0		00	0 (0)
Black bullhead	BEL-24	1	1	1	0	0	0	1	1	2 (8)
	HRL-30	0	0	0	0	0	0		00	0 (0)
	BAL-15	0	0	0	0	0	0		00	0 (0)
Mosquitofish	BEL-103	2	1	1	0	0	0	2	1	4 (4)
	HRL-90	0	0	0	0	0	0		00	0 (0)
	BAL-62	0	0	0	0	0	0		10	1 (2)
Total	BEL-511	8	2	4	0	0	2	29	5	35 (7)
	HRL-391	0	0	0	0	0	0	1	0	1 (1)
	BAL-354	0	0	0	0	0	0		20	2 (1)

^a Scientific names of fishes are given in Table 1.

^b BEL, Belevs Lake; HRL, High Rock Lake; BAL, Badin Lake.

^c A description of the characteristics of the abnormalities is given in the text.

^d Percentages were rounded to the nearest whole number.

^e Selenium inputs to Belevs Lake were curtailed in 1985.

species (numerically) with fathead minnows in 1982. The prevalence of abnormalities in Belevs Lake was higher in 1982 than in any of the other surveys, reaching 70% in green sunfish, 61% in common carp, and over 30% in fathead minnows and mosquitofish (Table 2). Skeletal deformities were found in adult fish as well as in juveniles. Although there were some species differences, the general pattern of abnormalities in 1982 had shifted back to that present in 1975, with spinal and fin deformities predominating. The reason(s) for this shift were not related to fish species composition, since none of the edema-prevalent planktivores had recolonized the lake. The occurrence of abnormalities in the reference lakes did not exceed 3%. The prevalence of abnormalities in Belevs Lake fish increased substantially in some species during the 1975–1982 interval but was essentially unchanged in others (Table 4). Fish from Belevs Lake contained from 82 to 120 $\mu\text{g/g}$ selenium, which was slightly less than that in the 1978 survey, but still elevated by as much as 131% over the 1975 levels (Tables 3 and 4). Fish from the reference lakes contained less than 3 $\mu\text{g/g}$ selenium (Table 3).

In 1992, largemouth bass, bluegill, and satfin shiners were present in Belevs Lake in addition to the six species collected in the 1982 survey. The two centrarchids had begun to recolonize the main body of the lake as individuals were gradually recruited from remote headwater areas. Satfin shiners were known to occur in Belevs Creek during the initial filling and stabilization of Belevs Lake prior to start-up of the power plant (1971–1973). Although not collected in previous surveys, satfins apparently

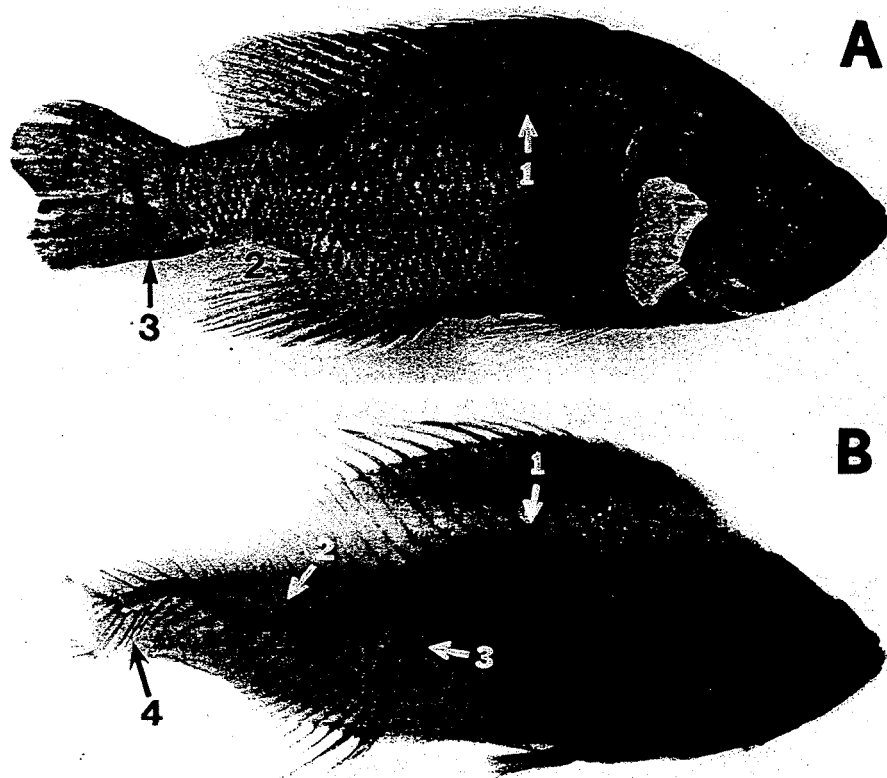


FIG 2 (A) External appearance of a deformed green sunfish (total length = 17.0 cm) from the 1978 Belews Lake survey. 1, kyphosis or "humped back" condition; 2 lordosis of the caudal peduncle region; 3, asymmetrical caudal fin due to deformed lower fin rays. (B) A-ray image of the same green sunfish: 1, kyphosis (convex curvature of the thoracic region of the spine); 2, lordosis (concave curvature of the lumbar and caudal regions of the spine); 3, compression of the ventral (pleural) ribs into the basipterygium of the anal fin; 4, compression of hemal spines near the tail.

exploited the predator-free environment of Belews Lake that persisted during the 1980s and became a numerically dominant cyprinid by 1992. The frequency of malformations was lower than that in the past, with all species having 11% or less (Table 2). Deformities were observed in adult fish as well as in juveniles. The pattern of abnormalities had shifted to predominantly fin defects followed by lordosis, cataracts, and kyphosis. As compared to 1982 levels, the prevalence of abnormalities in Belews Lake fish had dropped by 60 to 89% (Table 4). Selenium residues were generally below 20 $\mu\text{g/g}$ (Table 3) and had dropped some 76 to 86% from those of the previous survey in 1982 (Table 4). Although both the prevalence of abnormalities and selenium residues were sharply reduced in Belews Lake during the 1982–1992 interval, they were still 7 times (deformities) and 5 to 18 times (residues) the levels present in the reference lakes (Tables 2 and 3).

The relationship between whole-body concentrations of selenium and the frequency of malformations in the fish population of Belews Lake did not follow a clear trend

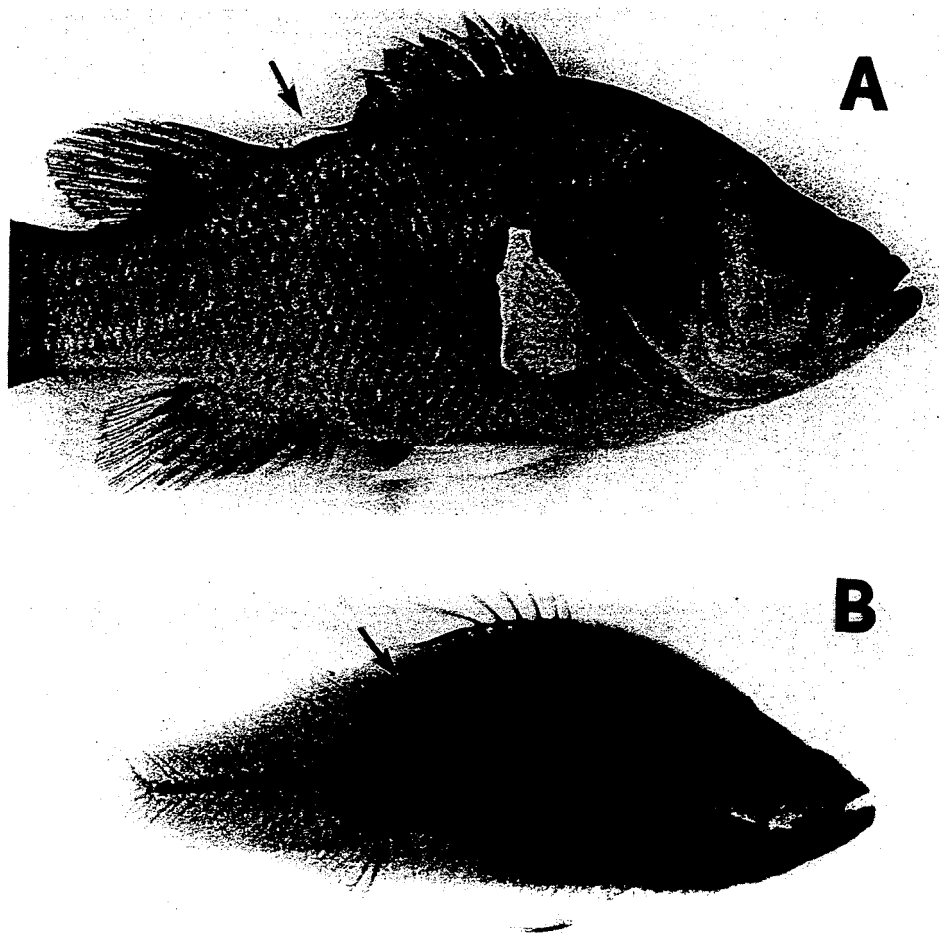


FIG. 3. (A) External appearance of another deformed green sunfish (total length = 14.2 cm) from the 1978 Belews Lake survey. This individual is missing part of the spinous dorsal fin (arrow). (B) X-ray image of the same green sunfish. Three hard dorsal spines and five supporting pterygiophores are missing from the skeleton (arrow).

when all species were considered together (Fig. 8). Malformations did not increase in direct proportion to increasing selenium, nor was there an apparent threshold or inflection point for the relationship. Rather, as selenium increased, the range in prevalence of deformities increased. The range of selenium residues was partitioned into three distinct, nonoverlapping groups with the vertical spread of each cluster (frequency of malformations) reflecting species and family differences (Fig. 8, Table 2). When only **centrarchids** were considered (Fig. 9) an association was identified. The relationship closely resembled an exponential function, with the inflection point or rapid rise in deformities occurring between 40 and 50 $\mu\text{g/g}$ selenium. The mathematical description of this relationship (polynomial regression, cubic model) indicated a good fit to the exponential function, with an R^2 value of 0.88 1 ($P < 0.01$).

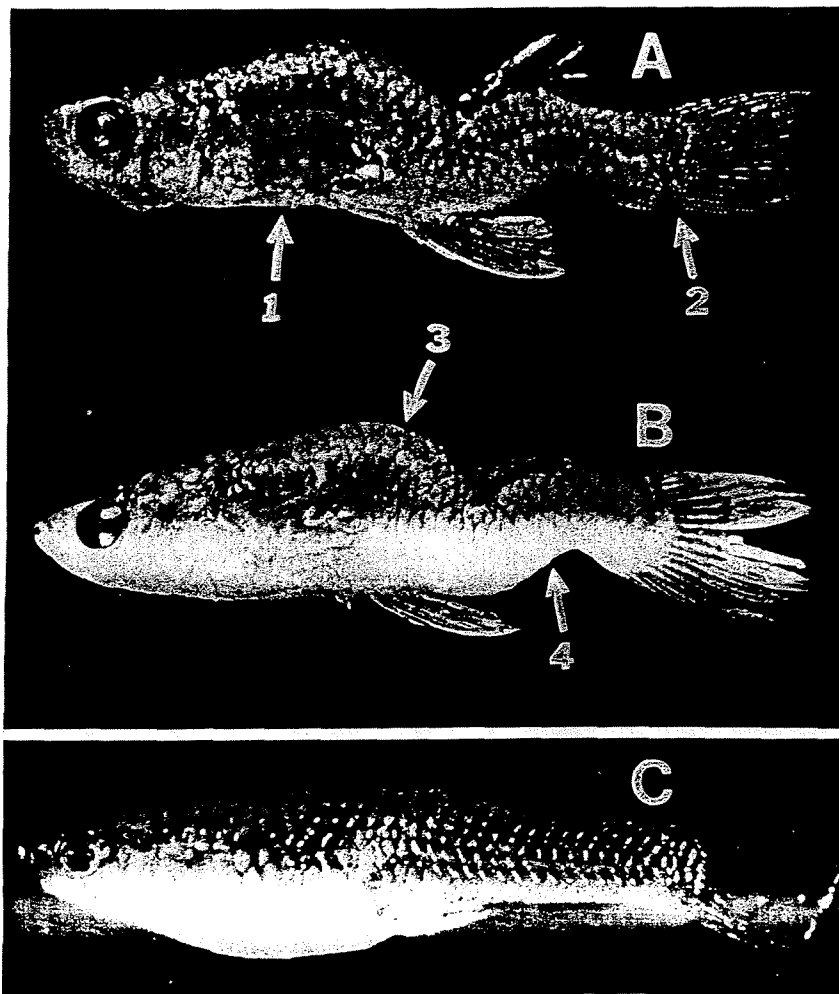


FIG. 4. Deformed (A and B; total lengths = 3.5 and 3.9 cm) and normal (C; total length = 4.1 cm) mosquitofish from the 1982 Belews Lake survey illustrating: 1, missing pelvic fins; 2, vestigial lower caudal fin rays; 3, kyphosis; 4, lordosis.

DISCUSSION

The pattern and types of abnormalities observed in fish from Belews Lake are classic symptoms of selenium toxicity. As early as 1937, Ellis *et al.* described edema and exophthalmus associated with chronic selenium poisoning in fishes. Several other laboratory and field studies have been conducted which document the occurrence of lordosis, kyphosis, **scoliosis**, and fin deformities in larval fishes resulting **from** parents that were exposed to levels of dietary and waterborne **selenium** similar to those in Belews Lake (Gillespie and Baumann, 1986; Woock *et al.*, 1987; Pyron and Beitinger, 1989; Schultz and Hermanutz, 1990; Hermanutz *et al.*, 1992). However, none of these experimental studies followed the survival of abnormal offspring into the juvenile and adult life stages because (1) by design, the studies were terminated at 10 to 30 days post-hatch or (2) the mortality of abnormal larvae was so complete by a few days past swim-up that there appeared to be no need to continue the experiments. In studies of

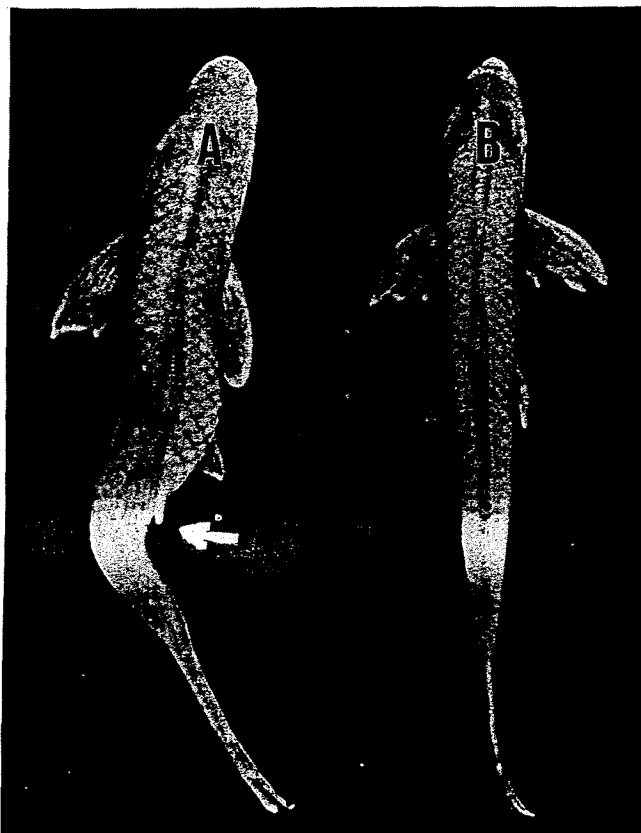


FIG. 5. Abnormal (A: total length = 8.3 cm) and normal (B: total length = 7.7 cm) red shiners from the 1982 Belews Lake survey illustrating deformity due to scoliosis, or lateral curvature of the spine (arrow).

rainbow trout, Goettl and Davies (1977) induced what they described as a deformity by exposing fertilized eggs to concentrations of waterborne selenium much higher than those in Belews Lake (80-1000 $\mu\text{g/L}$ vs 5-15 $\mu\text{g/L}$). Although the malformation persisted for the duration of a 13-month study, the only "deformity" observed was small, pointed heads. The apparent deformity was probably a growth response rather than a true teratogenic effect since the authors stated that cessation of growth occurred in the affected individuals. Hermanutz (1992) exposed fathead minnows to elevated selenium in outdoor experimental streams, allowed them to spawn, and then determined the incidence of malformations in offspring that survived to juvenile size (1.3 g). He found up to 30% deformity in these fish, thereby documenting that selenium-induced teratogenesis can persist well beyond the embryo-larval life stage in a predator-free environment.

There are likely two main reasons that deformed fish survived to juvenile and adult life stages in Belews Lake. First, selenium rapidly accumulated in the food chain and diet of adult fish as the power plant began its first year of operation in 1974 (Lemly, 1985a). This led to elevated selenium in the gonads of reproducing fish (Cumbie and Van Horn, 1978) which could have effectively flooded the 1974-1975 year classes with a very high percentage of abnormal larvae. Second, during this same time period dietary selenium was approaching levels that are lethal to the adults of sensitive species,

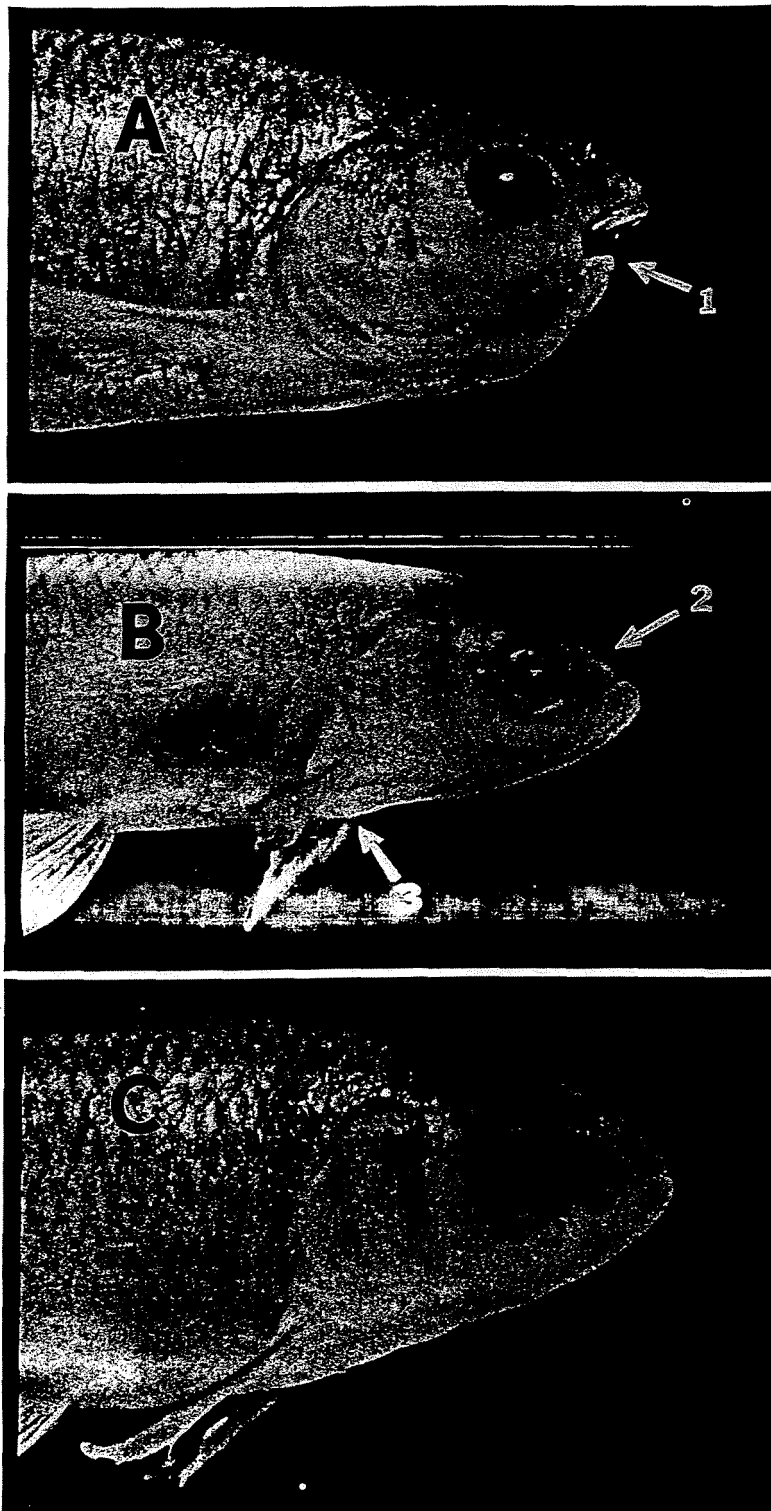


FIG. 6. Abnormal (A and B; total lengths = 7.9 and 6.5 cm) and normal (C; total length = 8.4 cm) red shinn from the 1982 Belews Lake survey showing 1, lower jaw and mouth deformities, 2, deformed head and olfactory nares (pughead); 3, vestigial pectoral fins.

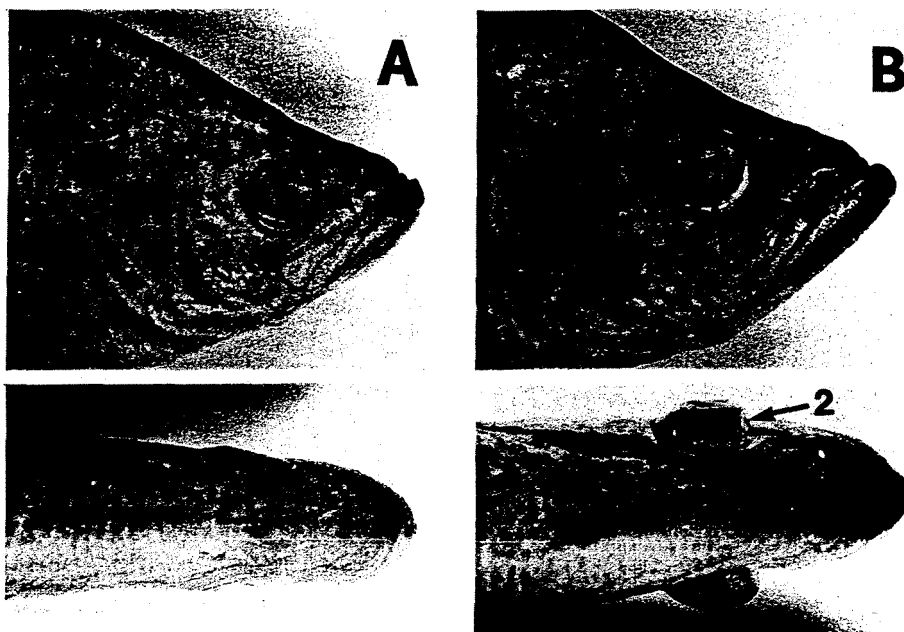


FIG. 7. Normal (A; total length = 16 cm) and abnormal (B; total length = 19.5 cm) white crappies from the 1975 Belevs Lake survey showing: 1, opaque cornea of the eye (cataract); 2, protruding eyeballs (popeye).

especially piscivores, and fish kills were observed (Harrell *et al.*, 1978). Elimination of some of the predation pressure on these deformed larvae may have contributed to their survival and eventual collection as juvenile fish in the 1975 survey of this study.

The levels of selenium present in the Belevs Lake food chain were sufficient to **eliminate** many of the original 19 species of fish within 3 years after the power plant began operating (Cumbie and Van Horn, 1978; Lemly, 1985a) and by 1978 virtually all of the piscivores and planktivores were gone. The deformed larvae of the remaining species had little competition or predation pressure and thus **experienced a** maximum probability of survival to adulthood. This was reflected in the high prevalence of deformities found in the 1978 and 1982 surveys. Selenium inputs from the power plant into Belevs Lake were curtailed in 1985. By 1992, concentrations of selenium in the food chain were low enough that some fish species had begun to recolonize the main reservoir. As compared to the other surveys, both the frequency of malformations and associated tissue residues of selenium had dropped sharply, *although* they **still persisted** at levels several times those in the reference lakes.

This study concentrated primarily on the identification of congenital malformations which appeared as structural aberrations visible through external inspection (**lordosis, kyphosis, scoliosis**, and head, fin, and mouth defects). These are true teratogenic effects, which include all morphologic, biochemical, and functional abnormalities produced before or at birth, and are generally considered to be irreversible (Fishbein, 1977; Harbison, 1980). None of the experimental selenium studies conducted to date have induced these types of abnormalities in juvenile or adult fish, which indicates that the defects observed in Belevs Lake fish must have originated as **embryolarval** deformities. Moreover, the true teratogenic defects were restricted to juvenile fish in the 1975 survey. The initial inputs of selenium into Belevs Lake (mid 1974 and early 1975) overlapped the period when these fish were spawned and explains why teratogenic

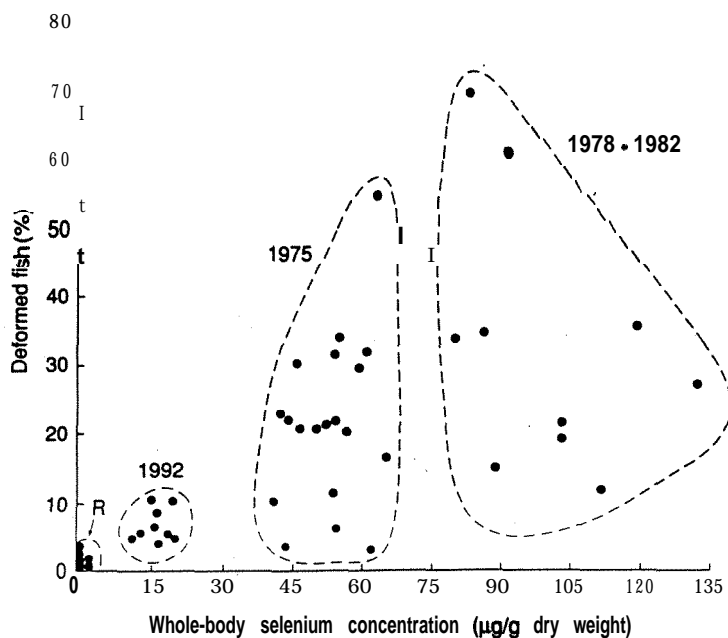


TABLE 3

WHOLE-BODY SELENIUM RESIDUES OF NORMAL AND ABNORMAL FISH FROM BELEWS LAKE
AS COMPARED TO THOSE IN NORMAL FISH FROM REFERENCE SITES

Year collected	Species ^a	Site ^b and number of samples analyzed ^c	Mean selenium concentration ^d	
			Normal fish	Abnormal fish
1975	White sucker	BEL-2	42.61	43.70
		HRL-1	1.83	—
		BAL-1	1.48	—
	Redbreast sunfish	BEL-2	58.36	56.12
		HRL-1	1.56	—
		BAL-1	1.02	—
	Green sunfish	BEL-2	66.89	65.19
		HRL-1	1.71	—
		BAL-1	1.47	—
	Pumpkinseed	BEL-2	46.74	48.34
		HRL-1	1.26	—
		BAL-1	1.01	—
	Warmouth sunfish	BEL-2	51.22	54.61
		HRL-1	1.06	—
		BAL-1	0.91	—
	Bluegill sunfish	BEL-2	53.83	50.97
		HRL-1	1.19	—
		BAL-1	1.05	—
	Redear sunfish	BEL-2	43.13	41.28
		HRL-1	1.94	—
		BAL-1	1.65	—
	Largemouth bass	BEL-2	59.22	58.40
		HRL-1	1.76	—
		BAL-1	1.28	—
	White crappie	BEL-2	62.37	60.21
		HRL-1	1.65	—
		BAL-1	1.03	—
	Black crappie	BEL-2	60.83	61.49
		HRL-1	1.69	—
		BAL-1	1.58	—
	Blueback herring	BEL-2	54.70	56.33
		HRL-1	1.94	—
		BAL-1	1.77	—
	Threadfin shad	BEL-2	39.84	44.96
		HRL-1	1.65	—
		BAL-1	2.14	—
	Common carp	BEL-2	63.32	62.11
		HRL-1	2.35	—
		BAL-1	2.01	—
	Golden shiner	BEL-2	46.54	48.37
		HRL-1	2.08	—
		BAL-1	1.73	—
	Black bullhead	BEL-2	57.29	56.07
		HRL-1	2.08	—
		BAL-1	1.73	—
	Channel catfish	BEL-2	60.91	66.10
		HRL-1	1.76	—
		BAL-1	1.82	—

TABLE 3-Continued

Year collected	Species ^a	Site* and number of samples analyzed ^c	Mean selenium concentration ^d	
			Normal fish	Abnormal fish
1978	White perch	BEL-2	55.01	54.63
		HRL-1	1.68	—
		BAL-1	1.94	—
	Yellow perch	BEL-2	41.87	44.72
		HRL-1	0.91	—
		BAL-1	1.16	—
	Mosquitofish	BEL-2	50.61	52.17
		HRL-1	1.21	—
		BAL-1	1.39	—
	Common carp	BEL-4	107.92	112.29
		HRL-2	2.44	—
		BAL-2	2.30	—
	Fathead minnow	BEL-4	86.97	80.13
		HRL-2	1.43	—
		BAL-2	1.85	—
1982	Black bullhead	BEL-4	94.18	103.05
		HRL-2	1.91	—
		BAL-2	1.60	—
	Mosquitofish	BEL-4	125.61	131.87
		HRL-2	2.06	—
		BAL-2	1.64	—
	Green sunfish	BEL-4	88.90	84.22
		HRL-2	1.91	—
		BAL-2	1.32	—
	Common carp	BEL-4	100.83	93.39
		HRL-2	2.94	—
		BAL-2	2.61	—
	Red shiner	BEL-4	85.44	89.20
		HRL-2	1.31	—
		BAL-0	—	—
1992 ^e	Fathead minnow	BEL-4	82.57	87.31
		HRL-2	1.49	—
		BAL-2	1.18	—
	Black bullhead	BEL-4	98.12	103.65
		HRL-2	2.03	—
		BAL-2	1.31	—
	Mosquitofish	BEL-4	117.26	120.47
		HRL-2	1.92	—
		BAG2	1.05	—
	Green sunfish	BEG2	12.40	14.68
		HRL-1	1.63	—
		BAL-1	1.19	—
	Bluegill sunfish	BEL-2	18.40	19.06
		HRL-1	1.67	—
		BAL-1	1.81	—
1992 ^e	Largemouth bass	BEG2	23.19	19.72
		HRL-1	1.34	—
		BAL-1	1.52	—
	Common carp	BEL-2	15.59	16.20
		HRL-1	2.85	—
		BAL-1	2.07	—
	Red shiner,	BEL-2	15.37	13.28
		HRL-1	1.26	—
		BAL-0	—	—

TABLE 3—Continued

Year collected	Species ^a	Site ^b and number of samples analyzed ^c	Mean selenium concentration ^d	
			Normal fish	Abnormal fish
	Satinfin shiner	BEL-2	12.39	11.17
		HRL-0	—	—
		BAL-1	1.33	—
	Fathead minnow	BEL-2	21.07	19.62
		HRL-1	1.16	—
		BAL-1	1.34	—
	Black bullhead	BEL-2	13.12	15.76
		HRL-1	1.91	—
		BAL-1	1.65	—
	Mosquitofish	BEL-2	18.90	16.48
		HRL-1	1.54	—
		BAL-1	1.62	—

^a Scientific names of fishes are given in Table I.

^b BEL, Belews Lake; HRL, High Rock Lake; BAL, Badin Lake.

^c Each sample was a composite of 3 to 5 individuals (6 to 10 individuals for mosquitofish, fathead minnows, and red shiners) except for 1992 Belews Lake abnormal fish samples, which were formulated from the total number of individuals collected.

^d Selenium concentrations in $\mu\text{g/g}$ (parts per million) on a dry weight basis.

^e Selenium inputs to Belews Lake were curtailed in 1985.

TABLE 4
TEMPORAL TRENDS IN SELENIUM RESIDUES AND ABNORMALITIES OCCURRING
IN THE FISHES OF BELEWS LAKE

Species ^a	Parameter ^b	Percentage change during interval	
		1975-1982	1982-1992 ^c
Common carp	AB	+58	-89
	SE	+48	-82
Green sunfish	AB	+15	-84
	SE	+31	-83
Fathead minnow	AB	0 ^d	-71
	SE	+9 ^d	-76
Red shiner	AB	— ^e	-60
	SE	—	-84
Black bullhead	AB	+12	-56
	SE	+84	-85
Mosquitofish	AB	+67	-89
	SE	+131	-86

^a Species other than these were eliminated from the lake during most of the study, preventing trend analysis; scientific names of the fishes are given in Table I.

^b AB, percentage occurrence of abnormalities; SE, whole-body selenium concentration.

^c Selenium inputs to Belews Lake were curtailed in 1985.

^d For 1978-1982 interval.

^e This species was introduced and became established in Belews Lake in 1980.

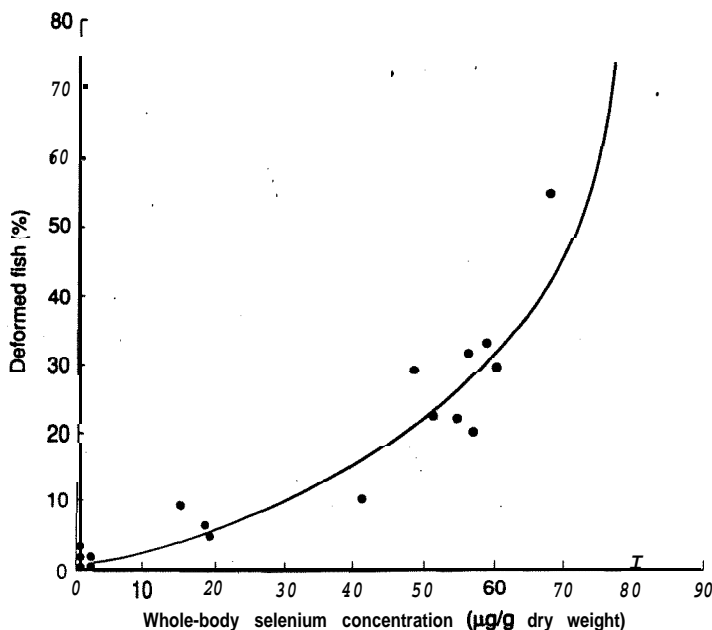


FIG. 9. Relationship between whole-body concentrations of selenium and frequency of deformities occurring in centrarchids from 1975 through 1992 (the R^2 value for a fit to the exponential function was 0.881, $P < 0.01$; cubic model). Each dot represents a different fish species.

Company, 1980; Finley, 1985; Woock *et al.*, 1987) or injecting them with selenium and holding them in high-selenium water (Ellis *et al.*, 1937). Edema and exophthalmus appear to be the result of an acute or subchronic toxic response in which selenium levels rapidly increase in the body and cause an accumulation of fluids in the body cavity and orbits. Cataracts are probably more of a chronic toxic response and may result from lower dietary and/or waterborne concentrations of selenium than those necessary to produce edema and exophthalmus. Selenium-induced cataract is a well-known condition in mammals and has been extensively studied and characterized histologically and biochemically (Shearer *et al.*, 1987). Results of the present study indicate that edema, exophthalmus, and cataracts can occur at a reasonably high frequency in natural fish populations exposed to elevated selenium.

The association between whole-body selenium concentrations and prevalence of deformities indicates a dose-response for the true teratogenic effects. A dose-response is expected for teratogenic substances (Harbison, 1980) and has been found to occur in laboratory selenium feeding studies with fish (Woock *et al.*, 1987). Of particular interest was the finding that the relationship approximated an exponential function when centrarchids were considered separate from other species. The inflection point for this function occurred at tissue residues of about 40 to 50 µg/g and a frequency of deformities between 20 and 30%. These values closely match the residues at which larval abnormalities exceeded 20% in experimental studies with bluegills (Bryson *et al.*, 1984; Giiespie and Baumann, 1986; Hermanutz *et al.*, 1992) and fathead minnows (Schultz and Hermanutz, 1990). The strength of the relationship ($R^2 = 0.881$, $P < 0.01$) suggests that selenium residues can be used to successfully predict the frequency of teratogenic defects in natural populations of centrarchids.

The abnormalities found in this study closely resemble those observed for populations of wild aquatic birds exposed to selenium in agricultural irrigation drainwater at Kesterson Reservoir in California (Ohlendorf et al., 1986, 1988; Kilness and Simmons, 1986; Presser and Ohlendorf, 1987; Hoffman et al., 1988; Ohlendorf, 1989). Both Kesterson Reservoir and Belews Lake represent aquatic habitats that were saturated with selenium to the point that direct mortality of adults occurred in addition to reproductive failure. The presence of multiple congenital malformities in fish, particularly deformed spines, heads, mouths, and fins, closely parallels the abnormal spines, beaks, heads, wings, legs, and feet observed for birds. Moreover, the frequency distribution of abnormalities among different species was quite similar and the maximum prevalence was very high in both groups (70% for fish, 65% for birds). It therefore appears that the teratogenic effects of selenium in natural populations of fish and aquatic birds are essentially the same.

This is the first detailed report of teratogenic effects of selenium in natural populations of freshwater fish. The only other field observations of possible selenium teratogenesis are those of Bryson et al. (1984) who published an X-ray image of a green sunfish with skeletal deformities that was collected from Hyco Lake, North Carolina. This impoundment is a power plant cooling reservoir which experienced selenium-related fishery problems similar to those in Belews Lake. No detailed studies of the incidence of deformities have been reported for Hyco Lake or other lakes and reservoirs where selenium accumulation has occurred. However, it is likely that deformed larvae and possibly juvenile and adult fish are present in these locations.

The situation in Belews Lake may have been somewhat unique because of the rate of selenium accumulation in the food chain and tissues of reproductively active fish (from $<2 \mu\text{g/g}$ to as much as $200 \mu\text{g/g}$ over the course of one spawning season), combined with a reduction in predation pressure on deformed larvae as adult fish succumbed to selenium poisoning. Belews Lake probably represents a best-case scenario for the survival of juvenile and adult fish with teratogenic defects. This set of circumstances would not necessarily be expected to occur in other locations or in other assemblages of fish species. It is more likely that selenium teratogenesis would produce deformed larvae that would die or quickly fall prey to piscivorous species and thus not be seen in the juvenile or adult populations. Nevertheless, the impact of teratogenesis on the fish community could be devastating since teratogenic defects are a very important component of selenium-induced reproductive failure (Gillespie and Baumann, 1986; Woock et al., 1987; Hermanutz et al., 1992).

Reproductive success is the most sensitive biological end point for assessing selenium poisoning in fish (Lemly, 1985a,b, 1992; Gillespie and Baumann, 1986; Schultz and Hermanutz, 1990; Coyle et al., 1992). One way to evaluate the importance of teratogenesis is to conduct ichthyoplankton surveys in locations known or suspected of having selenium-related problems with fish reproduction. Teratogenic effects could be detected and evaluated even if the affected individuals did not survive to become juvenile and adult fish. Without this type of assessment it is quite possible that selenium teratogenesis could occur and be an important part of reproductive failure, but go unnoticed.

CONCLUSIONS

The unique set of conditions in Belews Lake has allowed close examination of the toxicology of selenium in a field setting for nearly 2 decades. This aquatic system is

now in a recovery phase, with selenium levels and teratogenic **effects** continuing to decline. The studies of this reservoir have generated toxicity information that now forms the basis for national and state water quality criteria for selenium (U.S. EPA, 1987; North Carolina Department of Natural Resources and Community Development, 1986). There is a need to **continue** studies of this and other contaminated reservoirs to determine the length 'of time necessary for reestablishment of a reproductively active fish community with reference levels of selenium residues and **teratogenic** defects.

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